A STUDY OF HISTONE ACTION ON SYNTHESIS OF ANTIBODIES AND OTHER CELLULAR PROTEINS *IN VITRO*

Ye. V. Sidorova

NASA TT F-10,727

Translation of "Izucheniye deystviya gistonov na sintez antitel i drugikh kletochnykh belkov *in vitro*".
Biokhimiya, Vol. 31, pp. 789-798, 1966.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON D.C. FEBRUARY 1967

A STUDY OF HISTONE ACTION ON SYNTHESIS OF ANTIBODIES AND OTHER CELLULAR PROTEINS *IN VITRO*

Ye. V. Sidorova

(Laboratory of Chemistry of Antibody Biosynthesis, N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences USSR, Moscow)

The influence of different histones on the synthesis of antibodies and other proteins in rabbit's spleen cell culture during the secondary response *in vitro* has been investigated. The synthesis of antibody and non-specific γ-globulin was determined by measuring the specific increase of C^{14}-activity on immunosorbents; the synthesis of other water-soluble proteins -- as C^{14}-incorporation into TCA-precipitate.

It was shown that histones (20 - 30 γ/ml) inhibited the synthesis of antibodies of non-specific γ-globulins and of other proteins to 20 - 50%. The synthesis of antibody and of non-specific γ-globulin was inhibited at the same degree, but the formation inhibition of water-soluble protein was much less expressed. Different histone fractions possessed different inhibiting activity. The action of all histone fractions was proportional to its concentration. Low concentration of histones (5 - 10 γ/ml) in some cases stimulated the process of protein biosyntehsis *in vitro*. Maximal inhibition of antibody and non-specific γ-globulin synthesis was noted after 2 hours cultivation with histone. The decrease of other water-soluble protein formation appeared only after 4 hours of incubation. Tissue or species specificity of histones was absent. Total histones and lysine-histones from normal rabbit's spleen inhibited the protein synthesis stronger than the same fractions from the hypperimmune rabbit's spleen isolated on the 4th day after booster injection. On the contrary, lysine-histones from normal rabbit's liver were less inhibitory than lysine-histones from "immune" liver (isolated on the 4th day after boosting).

One of the most important questions in modern biochemistry is that of the /789* intracellular mechanisms regulating protein synthesis. In recent years, nuclear proteins -- and most of all, the histones (Ref. 1 - 3) -- have begun to attract more and more attention in this regard.

In studying the question of protein synthesis regulation in a model of antibody formation *in vitro*, we discovered that rabbit liver and spleen nuclei

---

* Numbers in the margin indicate pagination in the original foreign text.

contain certain proteins which drastically depress antibody biosynthesis (Ref. 4). The assumption arose that these substances were nuclear nucleoproteins, or histones. The present work was undertaken to verify this assumption.

Methods

Experiments were set up with chinchilla rabbits immunized with two antigens -- human serum albumin (HSA) and equine γ-globulin (EGG).

The test system used was a suspension of spleen cells removed on the fourth day after a reimmunization (booster shot). The cells were incubated at 37°C in Eagle's medium containing C^{14}-glycine (Ref. 5). The synthesis of antibodies to HSA (anti-HSA) and to EGG (anti-EGG) and of nonspecific γ-globulin was determined by the specific increment of radioactivity on proteocellulose copolymers (immunosorbents) (Ref. 5, 6). The synthesis of other cellular proteins soluble and insoluble in aqueous solutions was estimated by means of tags included in the precipitate formed when TCA (trichloroacetic acid) was added to the final concentration of 5%.

Deoxyribonucleoproteins (DNP) were separated by the method of McAllister et al. (Ref. 7) from whole tissues of the liver and spleen of normal and hyperimmune rabbits (four days after reimmunization). The DNPs separated were purified by triple redeposition with 1M NaCl at 0°C; part of the purified DNPs was in each case used for isolating the unfractionated histone (Ref. 7). From the remainder we separated histones rich in lysine (Ref. 8) and arginine (Ref. 9).

Preparations of histones dissolved in 0.9% NaCl with pH∿6 and sterilized by means of Seits filters were added to the specimens. Protein content in the histone preparations was determined by Louri's method and the nucleic acid content after hydrolysis in 0.5N $HClO_4$ was determined by Spirin's method (Ref. 10). It did not, as a rule, exceed 0.05-0.10%.

In order to exhaust the histones by the antigens (HSA and EGG), we utilized complexes of antigens with cellulose (C-HSA and C-EGG) (Ref. 11). For each 100 micrograms of histone we took 600 micrograms of antigen, i.e., 6 mg of sorbent, since the antigen comprises about ∿10% of the dry weight of the sorbents. Before exhaustion, the sorbent was set for 30 min. in 0.1N HCl and then washed 3 - 5 times with a physiological solution. To the washed sorbent residue, we added the required amount of histone, and the contents of the test tubes were carefully mixed at room temperature or at 37°C for 30 - 60 minutes. In the control experiments, an equal volume of 0.9% NaCl was added to the sorbents. Every 30 - 60 minutes the samples were centrifuged for 5 minutes at 10,000 g, and the supernatant fluid -- after sterilization by means of a Seitz filter -- was utilized in the experiments as the "exhausted" histone preparation.

Results of Investigation /790

The first series of experiments investigated the action of the DNPs

TABLE 1

INHIBITION OF THE SYNTHESIS OF ANTIBODIES AND OTHER CELLULAR PROTEINS BY DIFFERENT HISTONE FRACTIONS FROM SPLEEN AND LIVER OF NON-IMMUNIZED RABBITS

Histone Fraction (G)	Histone Concentration µg/cc	Protein Synthesis, imp/min/sample					Inhibition (-) or Stimulus (+) of Protein Synthesis, %				
		Anti-HSA	Anti-EGG	Nonspecific γ-Globulins	Other Water-Soluble Proteins	Insoluble Proteins, imp/min/mg	Anti-HSA	Anti-EGG	Nonspecific γ-Globulins	Other Water-Soluble Proteins	Insoluble Proteins, imp/min/mg
Spleen											
Control	—	121	682	1585	485	1070	—	—	—	—	—
Unfractionated	29	53	252	623	376	763	−56	−63	−60	−22	−21
Lysine-G	16	91	462	1112	424	1192	−25	−32	−30	−13	+11
Arginine-G	24	82	433	1071	463	862	−30	−36	−30	− 5	−19
Control	—	218	546	959	134		—	—	—	—	
Unfractionated	33	70	188	344	117		−68	−66	−65	−13	
Lysine-G	33	32	130	265	35		−85	−76	−72	−80	
Arginine-G	33	45	129	310	55		−79	−74	−68	−59	
Control	—	198	1122				—	—			
Unfractionated	33	169	805				−15	−28			
Lysine-G	33	115	580				−40	−49			
Liver											
Control	—	50	176	815	448	778	—	—	—	—	—
Unfractionated	41	46	132	529	291	812	− 8	−25	−35	−35	+4
Lysine-G	41	49	150	669	321	713	0	−15	−18	−29	−9
Arginine-G	23	59	114	514	285	773		−35	−37	−37	0
Control	—	152	469				—	—			
Unfractionated	37	37	110				−76	−77			
Lysine-G	34	92	253				−40	−44			
Arginine-G	37	52	100				−66	−78			
Control	—	678	1671	1875			—	—			
Unfractionated	22	183	412	631			−73	−75	−66		
Lysine-G	11	529	1742	—			−22	+ 4			
Control	—	139	289	1607	728		—	—	—	—	
Unfractionated	50	54	117	594	452		−60	−59	−63	−38	
" "	33	75	188	820	529		−44	−35	−50	−28	
" "	17	138	319	1354	596		0	+ 8	−16	−18	
" "	8	167	318	1497	1011		+20	+ 8	− 7	+40	
Control	—	198	1122				—	—			
Lysine-G	67	79	422				−60	−62			
" "	33	95	761				−50	−30			
" "	17	236	1134				+20	0			

isolated from nonimmunized rabbit spleen and liver upon protein synthesis. The triply precipitated spleen DNP inhibited antibody and nonspecific γ-globulin synthesis *in vitro*. The addition of 100 μg (with respect to protein) of DNP to the sample reduced synthesis of these proteins by 20 - 50%. In contrast to this, liver DNP barely depressed antibody and nonspecific γ-globulin formation -- and in a number of cases even stimulated the incorporation of tags. The formation of water-soluble proteins (not immunoglobulins) was not reduced under the effect of rabbit spleen DNP, but with the addition of liver DNP it was stimulated to a greater degree than was antibody synthesis.

The next series of experiments studied the action of histones isolated /791
from the liver and spleen DNP of nonimmunized rabbits.

When different histone fractions were added to spleen cells which actively synthesized antibodies *in vitro*, a sharp inhibition of antibody formation was observed (Table 1). Of the spleen histones, the lysine-histones had the stronger depressive action, while of the liver histones, it was the arginine-histones which had this action. The depressive action did not involve death of the cells -- the number of living and dead cells in the histone tests did not differ from these numbers in the control.

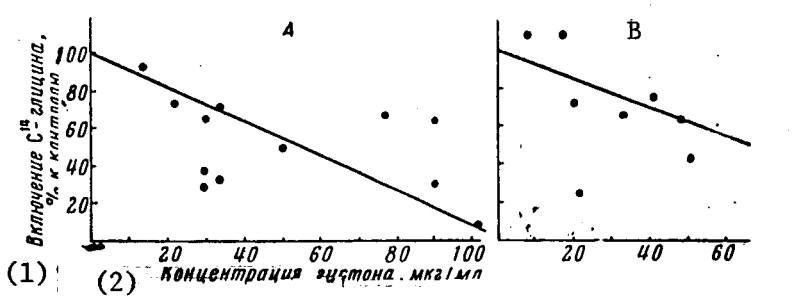

Figure 1

Dependence of the Influencing Unfractionated Rabbit-Liver and Spleen Histones Upon Their Concentration

A -- Synthesis of antibodies to equine γ-globulin on addition of unfractionated spleen histone; B -- Synthesis of antibodies to equine γ-globulin on addition of unfractionated liver histone.

(1) - C^{14}-glycine inclusion, % in comparison with control;
(2) - Histone concentration, μg/cc

The inhibiting effect was proportional to histone concentration in the medium. Figure 1 shows curves representing synthesis of antibodies to equine γ-globulin (EGG) as a function (derived in several experiments) of the concentration of unfractionated spleen and liver histones in the medium. Quite similar curves are also derived when determining the effect of histones on the synthesis of antibodies to HSA.

From a comparison of these curves the conclusion may be reached that the

unfractionated spleen and liver histones of nonimmunized rabbits have about the same depressive activity; a 50% repression of antibody formation is detected at a concentration of 50 - 70 μg/cc thereof. Similar curves were also obtained for the action of spleen and liver lysine-histones. Spleen lysine-histones caused a 50% inhibition of antibody synthesis at a 35 μg/cc concentration, and liver lysine-histones -- at a 60 μg/cc concentration.

It is interesting that in a series of experiments, small histone quantities (5 - 10 μg/cc) not only did not depress, but even stimulated, antibody synthesis. In individual cases, this stimulation reached 20 - 40% (Table 1, experiment 7).

The following control experiment was made to demonstrate the fact that the histones' inhibiting action is really caused by repression of antibody syntehsis and not by impairment of antibody capacity to combine with immunosorbents when they are extracted in the test. A culture liquid (2 cc) containing radioactive antibodies (anti-HSA) and anti-EGG) was incubated for 20 hours at 37°C with 100 μg of unfractionated spleen histone. Addition of this amount of histone to the cell suspension inhibited antibody synthesis by 66%. In the control experiment, the addition of 100 μg of histone exerted no effect on the degree of radioactivity elicited by specific sorbents. Thus, in the histone-free specimens this increment was 31 and 59 imp/min/specimen for anti-HSA and anti-EGG, respectively, and in the histone-containing specimens -- 32 and 62 imp/min/specimen. It is therefore obvious that histones do not affect the capacity of antibodies to combine with antigens. /792

Along with their repression of antibody synthesis, the different histones inhibited formation of nonspecific γ-globulins to the same degree. As is evident from Table 1, these processes are completely parallel. Synthesis of the remaining water-soluble proteins of spleen cells, on the contrary, was inhibited to a substantially less degree, particularly at low histone concentrations (Table 1). In the majority of cases, during the action of unfractionated spleen histone, water-soluble protein synthesis was 20 - 50% higher after 20 - 24 hours of incubation than was antibody and unspecific γ-globulin synthesis.

The differences in histone action on specific and nonspecific γ-globulin synthesis *in vitro*, on the one hand, and on the synthesis of the remaining water-soluble spleen-cell proteins, on the other, are particularly apparent when these processes are traced over a period of time. Figure 2 gives the characteristic curves representing the formation of anti-EGG, nonspecific γ-globulin, and other water-soluble proteins after 2, 4, 8, and 20 hours of incubation with unfractionated histones of normal rabbit spleen and liver. As is evident, synthesis of antibodies and nonspecific γ-globulins is reduced even in the first two hours of incubation with histone. In these periods the synthesis of water-soluble proteins in the histone tests proceeds with the same intensity as in the tests without histone. Reduction in water-soluble protein syntehsis as a rule becomes appreciable only after 4 hours of incubation. In the latter periods the inhibition of protein synthesis is not increased, and at times even decreases. The reduction of inhibitory action is particularly noticeable in the experiments with unfractionated spleen histones. As already noted, even after 24 hours of incubation the synthesis of water-soluble proteins remains 20 to 50% higher than that of antibodies.

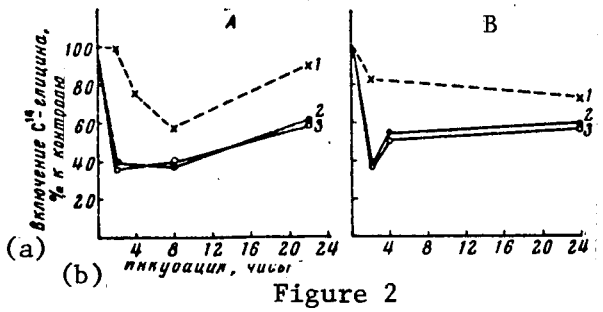

Figure 2

Inhibition of Protein Synthesis by Unfractionated Rabbit Spleen Histone (A) and Unfractionated Rabbit Liver Histone (B) in Different Periods of Incubation

1 -- C^{14}-glycine incorporated in water-soluble proteins,
2 -- in anti-EGG, 3 -- in nonspecific γ-globulins.

(a) - Inclusion of C^{14}-glycine in terms of % of control;
(b) - Incubation, hr.

In a special series of experiments, histone specificity was studied by animal species. For this purpose, unfractionated histones isolated from the spleen of chickens, rats, pigs, dogs, and rabbits were added to spleen cells synthesizing antibodies. Table 2 displays the results obtained. As is clear from Table 2, all the histones tested had a more or less repressive action and inhibited antibody synthesis by 30 - 70%. /793

It was of particular interest to find out whether any difference existed between the action of histones isolated from the organs of normal and immune rabbits. This series of experiments studied unfractionated histones and histones rich in lysine isolated from the spleen and liver of normal rabbits and from the same organs of animals on the fourth day after reimmunization -- i.e., during the period of maximum antibody synthesis. Table 3 summarizes the findings, and Figures 3 - 5 represent them graphically. It was found that unfractionated histones and lysine-histones from the spleen of immune rabbits (3 to 5 spleens were used) have a somewhat less repressive effect than do histones from the normal animal spleens. At equal concentrations, antibody synthesis inhibition by unfractionated histones from "normal" and "immune" spleens was 66 and 56%, respectively, in one of the experiments, and 70 and 20% in another. Lysine-histones from "normal" and "immune" spleen depressed antibody formation respectively by 80 and 36%.

Differences between the action of normal and immune spleen histones were also detected with respect to their depression of the synthesis of nonspecific γ-globulins and other water-soluble proteins. The histones from organs of immunized animals (particularly lysine-histone) possessed considerably less inhibiting action. It is interesting that the lesser depressive effect of the unfractionated histone from the immune spleen was also apparent in

TABLE 2

COMPARISON OF EFFECT EXERTED BY UNFRACTIONATED HISTONES ISOLATED FROM SPLEENS OF VARIOUS SPECIES OF ANIMALS ON ANTIBODY SYNTHESIS IN A SUSPENSION OF HYPERIMMUNE RABBIT SPLEEN CELLS *IN VITRO*

Concentration of histones -- 33 μg/cc; specimen volume, 3 cc; tissue content, 122 cc per specimen. Each number pertains to the mean of three specimens.

Animal Species	Antibody Synthesis, imp/min/specimen		Antibody Inhibition, %	
	Anti-HSA	Anti-EGG	Anti-HSA	Anti-EGG
Rabbit	61	181	-30	-26
Chicken	49	127	-44	-48
Rat	61	157	-30	-36
Pig	25	72	-71	-71
Dog	39	77	-55	-68
Control (no additions)	87	244	-	-

relationship to inhibition of nonspecific γ-globulin synthesis *in vitro* by non-immunized rabbit spleen cells. Thus, unfractionated histone from normal spleen inhibited synthesis of nonspecific γ-globulins by 43%, while histone from immune spleen did so by 28%.

/794

There were also definite differences in the action of histones from "normal" and "immune" liver (taken on the fourth day after reimmunization), which were directly contrary to those observed in spleen histones. Thus, lysine-histone isolated from "immune" liver inhibited synthesis of all proteins studied substantially more intensely than did normal liver lysine-histone (Figure 5). A 50% depression of synthesis was reached at a concentration of "immune" lysine-histone as low as 20 μg/cc, while "normal" lysine-histone in this dosage had practically no effect. No less than 60 μg/cc of normal liver lysine-histone had to be added to achieve a 50% inhibition of protein synthesis. /795

As in the case of unfractionated histones from normal tissues, it was demonstrated that "immune" liver lysine-histone does not affect the capacity of antibodies and nonspecific γ-globulins to combine with the corresponding sorbents, but inhibits their synthesis.

Proceeding from the supposition that the antigen may specifically eliminate (or bind) any of the histone fractions and in this process depress specific antibody formation, we made an attempt to remove the antibody synthesis-inhibiting action of normal spleen histones by exhausting them with the

TABLE 3

DIFFERENCE IN ACTION OF UNFRACTIONATED HISTONES AND LYSINE-HISTONES FROM LIVER AND SPLEEN OF NONIMMUNIZED RABBITS ("NORM") AND RABBITS 4 DAYS AFTER REIMMUNIZATION ("IMMUNE")

Histone (G) Fraction	Histone Concentration, μg/cc	Inhibition (-) or Stimulation (+) of Synthesis, %			
		Anti-HSA	Anti-EGG	Non-specific γ-globulins	Other Water-Soluble Proteins
Spleen					
Unfractionated "norm"	33	-68	-66	-65	-13
Unfractionated "immune"	33	-56	-56	-50	-20
Unfractionated "norm"	29	-68	-70	-67	-69
Unfractionated "immune"	29	-42	-20	-36	-31
Lysine-G "norm"	33	-85	-76	-72	-80
Lysine-G "immune"	33	-36	-36	-33	+ 3
Liver					
Unfractionated "norm"	22	-73	-75	-66	
Unfractionated "immune"	22	-75	-74	-64	
Unfractionated "immune"	22	-68	-64	-58	
Lysine-G "norm"	17	+20	0		
Lysine-G "immune"	17	-60	-60		
Lysine-G "norm"	11	-22	+ 4		
Lysine-G "immune"	7.3	-65	-62		

corresponding antigens. For this purpose, the histones were incubated with C-HSA and C-EGG for 30 - 60 minutes, and then these "exhausted" histones were added to specimens of spleen which synthesized antibodies. In no case, however, could the depressive histone action be removed by "exhaustion" (Table 4).

Discussion of Results

The idea that protein synthesis begins because of depression of the genes (Ref. 12) has drawn attention to the question of the nature of the specific repressors. Since the time that Stedman enunciated his hypothesis that nuclear histones perform this function, eighteen years have passed (Ref. 13), but there are a few data on the action of histones on the organism *in vivo* (Ref. 14) and somewhat more numerous data on histone action upon various isolated enzymatic systems (Ref. 15, 16) or subcellular fractions (Ref. 17 - 19). We know of only a single work devoted to a study of the effect of histones on protein synthesis in mammalian cells *in vitro* (Ref. 20), but investigations

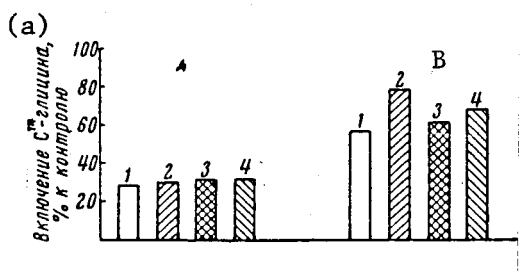

Figure 3

Inhibition of Antibody and Other Cell-Protein Synthesis by Unfractionated Histones of Normal Rabbit Spleen (A) and of Spleen Removed on the 4th Day After Reimmunization (B).

Histone concentration 29 g/cc. 1 -- Inclusion of C^{14}-glycine in anti-HSA, 2 -- in anti-EGG, 3 -- in nonspecific γ-globulins, 4 -- in other water-soluble proteins.

(a) - C^{14}-glycine inclusion in % of control.

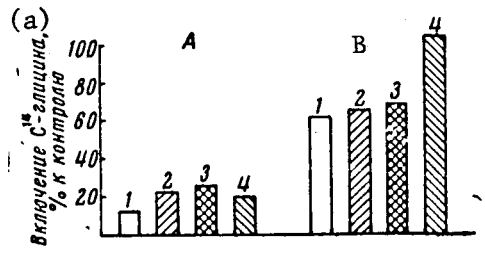

Figure 4

Inhibition of Antibody and Other Cell-Protein Synthesis by Lysine-Histones From Normal Rabbit Spleen (A) and from Spleen Removed on the 4th Day After Reimmunization (B).

Lysine-histone concentration 33 μg/cc. Notation is the same as in Figure 3.

(a) - C^{14}-glycine inclusion in % of control.

of this type are of definite interest.

We felt it was advisable to study this with a model of antibody synthesis *in vitro*. As a result, it was shown that histones depress protein synthesis not only in subcellular granules, but also in entire cells. Thus, individual fractions of histones from rabbit spleen and liver reduce the formation of antibodies and other cellular proteins in a suspension of the spleen of reimmunized rabbit in a concentration as low as 10 - 14 μg/cc. The depressive effect is proportional to histone concentration in the medium, and is most clearly apparent in the early incubation periods.

It is characteristic that histones, like some other factors which we have studied (Ref. 4), impair the synthesis of antibodies and nonspecific γ-globulins much more rapidly than that of other cellular proteins. The mechanism of this effect has hitherto been quite obscure. This is possibly associated with the fact that the γ-globulin molecule is an "agglomerate" of two types of chains, which increases the vulnerability of the process.

It should be noted that there is a strict parallelism between the degree of inhibition of antibody and nonspecific γ-globulin synthesis. This seems to us to be an additional argument in favor of the earlier enunciated hypothesis

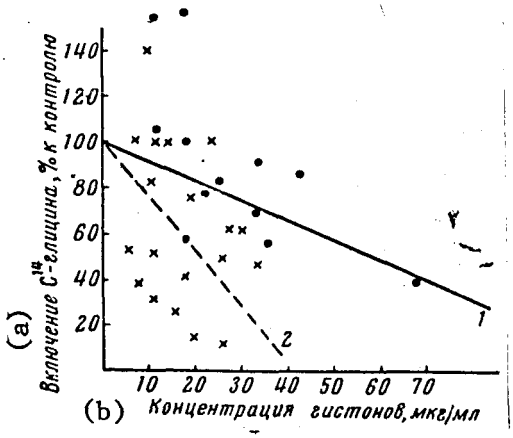

Figure 5
Comparison of Inhibiting Effect Exerted by Lysine-Histone from "Normal" Rabbit Liver and from Liver Removed the 4th Day After Immunization Upon Synthesis of Antibodies to EGG.

1 -- Inclusion of C^{14}-glycine in antibodies on adding lysine-histones of "normal" liver (dots indicate individual experimental findings), 2 -- Inclusion of C^{14}-glycine in antibodies on adding lysine-histones of "immune" liver (crosses indicate individual experimental findings).

(a) - Inclusion of C^{14}-glycine in % of control; (b) - Histone concentration, μg/cc.

of simultaneous synthesis of antibodies and nonspecific γ-globulins in the very same cell (Ref. 21).

All the data obtained indicate that histones act as non-specific inhibitors of protein synthesis in the cell. The lack of specificity is manifested in the fact that the histones inhibit synthesis of the most diverse proteins (even including such highly specialized ones as antibodies), and in the fact that histones extracted from different rabbit organs -- or even from different species of animals -- possess a depressive effect. It is possible that the lack of specificity may merely be illusory. Our /796 experiments employed extremely heterogeneous histone fractions whose effect varied from experiment to experiment, and the quantitative differences may have completely masked the qualitative differences. In order to reach definite conclusions, further tests must be conducted with uniform histone fractions purified to the maximum.

Against the background of the general, nonspecific, histone inhibiting effect on protein synthesis, we were able to detect a difference in the action of histones from the organs of normal and hyperimmune rabbits. This was particularly apparent in the tests with the liver lysine-histones.

As has been found, lysine-histones isolated from the liver on the fourth day after rabbit reimmunization have a significantly greater inhibiting effect on protein synthesis than do lysine-histones from nonimmunized animal liver. This is all the more interesting because, as is well known, the liver does not participate in the formation of γ-globulins and antibodies (Ref. 22). Campbell and Harvey's findings on the prolonged storage of antigen fragments in the liver (Ref. 23) led to the assumption that the effect of "immune" lysine-histone is caused by the presence of an antigen in this fraction. Lysine-histone added to already synthesized C^{14}-antibodies did not, however, impede their uniting with specific immunosorbents, which would have happened if lysine-histone had contained an antigen. Moreover, when added to spleen cells *in vitro*, lysine-histone drastically reduced not only antibody synthesis in them, but also synthesis of nonspecific γ-globulins and of other water-soluble proteins -- i.e., it acted as a nonspecific repressive agent. The action of lysine-histone from the liver of reimmunized rabbits therefore is not connected

TABLE 4

EFFECT EXERTED BY "EXHAUSTED" AND "UNEXHAUSTED" NORMAL LIVER HISTONES ON ANTIBODY SYNTHESIS

Histone (G) Fraction	Histone Concentration, µg/cc	Synthesis Inhibition, %	
		Anti-HSA	Anti-EGG
Unfractionated	33	-15	-28
Unfractionated exhausted EGG	33	-10	-13
Unfractionated	29	-72	-70
Unfractionated exhausted EGG	29	-68	-62
Unfractionated	33	-50	-50
Unfractionated exhausted HSA	33	-55	-50
Unfractionated	32	-	-30
Unfractionated exhausted HSA	32	-	-50
Lysine-histone	29	-24	-
Lysine-histone exhausted EGG	29	-34	-

with the presence of antigen or of fragments thereof in this fraction. The reduction in the amount of C^{14}-proteins discovered under the effect of lysine-histones is caused by impairment of their synthesis anew.

The greater inhibiting action of unfractionated histone and lysine-histone from nonimmunized rabbit spleens, in comparison with similar fractions from the spleens of hyperimmune animals, led us to assume that with immunization, the antigen specifically eliminated or bound a portion of the histones and thus "derepressed" antibody synthesis. Attempts to extract this hypothetical specific histone fraction with antigens in our experiments, however, proved to be unsuccessful. Moreover, inhibition of water-soluble protein synthesis by histones from "nonimmune" spleens was also greater than in the experiments with histones from "immune" spleens. This indicates the absence of the assumed specificity.

The difference in the action of histones from organs of nonimmunized and immunized rabbits is possibly caused by changes in the ratios of the various histone subfractions in the preparations which we derived. These relationships may change both specifically (in response to immunization) and nonspecifically as the result of some other processes -- for example, changes in activity of proteolytic enzymes which degrade the histones in the extraction process, changes in the extractability of this or that subfraction, etc.

Also important is the question of the mechanism of histone action upon

the cell. The findings made up to the present time permit us to assume at least three possibilities: (1) impairment of osmotic equilibrium in the cells because of the histones' combining with the proteins in the cell wall, plus a change in its penetrability leading to impairment of various cell functions, including protein synthesis. Data of this sort were derived in experiments with certain bacteria [Ref. 24]). (2) Combination with nuclear DNA and blockage of the formation of different ribonucleic acids in it, and thus also protein synthesis (Ref. 2, 3). (3) Binding of cytoplasmic ribonucleic acids and impairment of their activity (Ref. 25).

The experiments which we have conducted do not enable us to decide which /797
of these mechanisms takes place, nor is the fact excluded that the mechanism of inhibitory action on the part of different histone fractions is not the same. It is probable that a certain role in the process of protein synthesis repression is played by the free charged groups of histone. Blocking of these groups -- e.g., by DNA -- in a number of cases eliminates the histone's depressant effect.

In summarizing all the statements presented above, we must stress the fact that a study of histone action on the cellular level will possibly enable us to detect differences in the structure and functions of individual histone fractions, on the one hand, and to develop some methods of regulating the synthesis of proteins in the cell (including antibodies) on the other.

Conclusions

The addition of immune rabbit histones to spleen cells inhibits the *in vitro* formation of the antibodies, nonspecific γ-globulins, and other water-soluble proteins. Here the synthesis of nonspecific γ-globulins is repressed to the same degree, while the synthesis of water-soluble proteins is inhibited to a substantially less degree (20 to 50%) than is antibody synthesis. The different histone fractions possess varying depressant activity. With histone concentrations of 30 to 60 μg/cc, a 50% inhibition of antibody and nonspecific γ-globulins is achieved. A histone concentration on the order of 5 to 10 μg/cc in a number of cases stimulates protein synthesis.

Maximum repression of antibody and nonspecific γ-globulin synthesis is observed two days after incubation with histone. Inhibition of synthesis of other water-soluble proteins appears no earlier than after 4 hours of incubation.

Histone specificity with regard to tissue or species could not be detected.

The action of unfractionated histones and lysine-histones of normal rabbit liver and spleen is different from that of identical fractions isolated from organs of hyperimmune rabbits on the fourth day after reimmunization. Unfractionated histones and lysine-histone of normal spleen possess a greater repressant effect, and the lysine-histone from normal liver has less of a repressant effect, than the corresponding histones from the liver and spleen of immune animals.

The antibody-synthesis inhibiting action of histones from normal rabbit spleen cannot be eliminated by exhaustion with the corresponding antigens.

I am deeply grateful to A. Ye. Gurvich for discussing the paper.

REFERENCES

1. Butler, J. A. V. Nature, 207, 1041, 1965.
2. Busch, H. Histones and Other Nuclear Proteins. Acad. Press, N. Y., 1965.
3. The Nucleohistones. (Proc. 1st World Conference on Histone Biology and Chemistry, 1963.) 1964.
4. Gurvich, A. Ye., Sidorova, Ye. V., Tumanova, A. Ye., Hsü Fen. Biokhimiya, 30, 1044, 1965.
5. Gurvich, A. Ye., Sidorova, A. Ye. Ibid., 29, 556, 1964.
6. Drizlikh, G. I., Gurvich, A. Ye. Ibid., 29, 1054, 1964.
7. McAllister, H. C., Wan, Y. C., Irvin, J. L. Analyt. Biochem., 5, 321, 1963.
8. De Nooij, E. H., Westenbrink, H. G. K. Biochim. et Biophys. Acta, 62, 608, 1962.
9. Reid, B. R., Cole, R. D. Proc. Nat. Acad. Sci. USA, 51, 1044, 1964.
10. Spirin, A. S. Biokhimiya, 23, 656, 1958.
11. Gurvich, A. Ye., Kuzovleva, O. B., Tumanova, A. Ye. Ibid, 27, 246, 1962.
12. Jacob, F., Monod, J. J. Mod. Biol., 3, 318, 1961.
13. Stedman, E., Stedman, E. Cold Spring Harbor Symp., 12, 224, 1947.
14. Hnilica, L. S., Holoubek, V. Brit. J. Cancer, 15, 687, 1961; Nature, 191, 992, 1961.
15. Person, P., Fine, A. Science, 132, 43, 1960.
16. Gurley, L. R., Irvin, J. L., Holbrook, D. J. Biochem. and Biophys. Res. Communs, 16, 527, 1964.
17. Rivenbark, W. L., Hanson, J. B. Biochem. and Biophys. Res. Communs, 7, 318, 1962.
18. Hirschbein, L., Rozencwaig, R., Khouvine, Y. Compt. Rend., 255, 593, 1962.
19. Allfrey, V. G., Mirsky, A. E. Cold Spring Harbor Symp., 28, 247, 1963; Proc. Nat. Acad. Sci. USA, 49, 414, 1963.
20. Becker, F. F., Green, H. Exptl. Cell Res., 19, 361, 1960.
21. Sidorova, Ye. V. Voprosy Meditsinskoy Khimii, 11, 89, 1965.
22. Miller, L. L., Bly, C. G., Bale, W. J. Exptl. Med., 99, 133, 1954.
23. Campbell, D. H., Garvey, J. S. Lab. Invest., 10, 1126, 1961; J. Exptl. Med., 105, 361, 1957.
24. Guillaume, J., Derieux, J. C., Martin, G. et al. Ann. Inst. Pasteur, 13, 1, 1962.
25. Lallier, R. Compt. Rend., 256, 5409, 1963.

Received
November 23, 1965

Scientific Translation Service
4849 Tocaloma Lane
La Canada, California